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THE EFFECT OF INTERFERON ON THE INHIBITION OF SYNTHESIS  
OF PROTEINS IN A CULTURE OF CHICK EMBRYO  
FIBROBLASTS INOCULATED WITH ARBOR VIRUS

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Inhibition of synthesis of protein in the cell infected by a virus has been demonstrated in respect of a number of viruses and cells. It has been suggested (1) that this effect is responsible for destruction of cells. The influence of interferon on the inhibition of protein synthesis in virus-infected cells has been investigated (2) in a Mungo virus - L cells system, but it had no detectable effect on the decline of synthesis of protein.

The present article reports the influence of interferon on the inhibition of protein synthesis in a culture of chick embryo fibroblasts following infection with the virus of Venezuelan equine encephalitis (VEE).

Methods

Use was made of a strain of the VEE virus from the virus museum of the Ivanovskii Institute of Virology, obtained as a clone by isolation from a plaque twice.

A culture of chick embryo fibroblasts was developed in Carrel's dishes (diameter 60 mm). The culture medium consisted of 0.5% lactalbumin hydrolysate in Hanks' solution containing 10% ox serum. The dish was treated with 10 ml of medium containing 600,000 cells per ml. In 48 hr the cells formed a monolayer consisting of 4-5 million cells in each dish.

To infect cultures the medium was removed and 0.5 ml of virus-containing material was added to the culture. After adsorption for 2 hr at 4°C the culture was washed three times with Hanks' solution and suspended in 2 ml of medium no.199 containing 2% ox serum. After incubation for 6 hr at 37°C the medium was collected and stored at 4°C. The virus titer ranged from  $4 \times 10^9$  to  $6 \times 10^9$  of plaque-forming units (PFU) per ml. For titration the virus was mixed with medium no.199 containing 2% ox serum and adsorption was performed by the above-described method, and then the dishes were loaded with agar to the specification of Porterfield and Allison (4) as modified by us (3). Plaques were counted after 48 hr incubation at 37°C.

To assess the synthesis of protein the cells were removed from the glass with versene at various intervals after infection and suspended in a ml of Hanks' solution, and 0.2 ml of radioactive hydrolysate of Chlorella (label  $C^{14}$ , EuC per ml) was added. After incubation for 15 min at 37°C trichloroacetic acid was added to 5% concentration. The deposit was washed off by a technic described earlier.

Radioactivity was determined with a BFL counter having a counting efficacy of 4%. As control for non-specific adsorption use was made of cells pretreated with 0.05 M sodium azide.

Interferon, which was kindly supplied by T. I. Balezina, was titrated by exposing the cultures to its various dilutions for 24 hr at 37°C and then the cultures were infected with VEE virus at the rate of 100 PFU per dish and flooded with agar. A unit of interferon was the dose which decreased the plaque count to 50% of the control number. The preparation employed was found to contain 5120 units per ml.

#### Results of the Investigations

Within 3 hr after infection with VEE virus, protein synthesis in the chick embryo fibroblasts fell to 20-25% of the initial rate (Fig. 1). The density of infection (in the range of 5-400 PFU per cell) did not significantly affect the curve of decline of protein synthesis.

To study the influence of interferon on the depression of protein synthesis, the culture was, after 24 hr pretreatment with interferon, infected with the virus and again incubated with the same concentration of interferon. Treatment of the culture with interferon at a concentration of 32 units per ml did not alter the course of decline of protein synthesis after infection (Fig. 1). This dose of interferon did not protect the cells against a cytopathic effect despite the fact that the yield of virus decreased steeply (see table). A 100-unit dose of interferon likewise did not prevent the development of the cytopathic effect although it completely blocked the multiplication of the virus (see Table).

An entirely different picture was seen in cells exposed to a 1000-unit dose of interferon. Their synthesis of protein did not decline after infection with the virus (Fig. 1) and a cytopathic effect did not develop. The fall of protein synthesis failed to occur not only 3-4 hr but also 24 hr after infection, although at this

time, with a very high density (500 PFU per cell), slight cytopathic changes developed (Fig. 2).

#### Discussion

The findings not only show that the decline of protein synthesis following virus infection can be prevented by treatment with interferon but also suggest probable mechanisms of this effect.

The simplest and most likely explanation would be that in the system in question (in contrast to the Mungo-L system (2) there is in the interferon-treated cells a direct block of the formation of the viral "early protein" which causes a decline of protein synthesis in the cell (5). One should, however, bear in mind another possible explanation. In this system the decline of protein synthesis did not begin until 1½-3 hr after infection, i.e., after the start of replication of viral RNA. Therefore, the described effect may be an indirect result of block of synthesis of viral RNA by interferon.

If this latter assumption were correct one would expect that an increase in the amount of "extraneously introduced" viral RNA (i.e. increase of density of infection) would lead to "breakdown" of the protective action of interferon and protein synthesis in the cells would decline. This does not, however, occur, even when the infecting dose is increased to 500 PFU per cell. Further, if the described effect of interferon were due solely to block of viral RNA replication, one would expect a high correspondence between the dose of interferon which blocks the multiplication of the virus and the dose which prevents the development of the cytopathic effect.

Thus, the more probable mechanism of the described effect would be a direct inhibition of formation of a factor which inhibits the synthesis of cellular protein.

### Summary

1. In chick embryo fibroblasts infected with the virus of Venezuelan equine encephalitis the rate of protein synthesis fell to 20-25% of the initial level within 3 hr after infection.

2. In cultures treated with high concentrations of interferon a subsequent infection failed to cause a decline of protein synthesis not only 3-4 but also 24 hr after infection. The cytopathic effect either did not appear or else (at an infection density of 500 PFU per cell) developed only to a minor degree.

3. Lower concentrations of interferon (32-100 units per ml) inhibited multiplication of the virus but did not prevent the decline of protein synthesis and the development of the cytopathic effect.

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Fig. 1. The influence of interferon on the decline of synthesis of protein in chick embryo fibroblasts which have been infected with VEE virus.

Along vertical: rate of protein synthesis (as % of normal); along horizontal: time in hr ; 1 = cells not pretreated with interferon; 2 = dose of interferon 32 units per ml; 3 = dose of interferon 1000 units per ml.

(Table of p. 29)

Influence of interferon on multiplication of VEE virus  
and development of cytopathic effect.

Dose of interferon (units per ml)	Density of infection	Yield of virus	Cytopathic effect 24 hr after infection
	in PFU per cell		

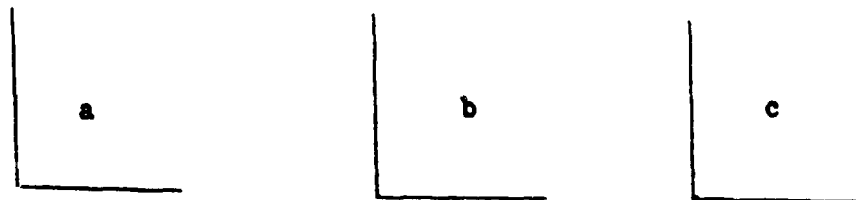


Fig. 2. Influence of interferon on cytopathic effect caused  
by VEE virus in culture of chick embryo fibroblasts.  
a = normal culture; b = culture pretreated with 1000 units interferon  
and infected with VEE virus (500 PFU per cell); c = culture not treated  
with interferon 24 hr after infection with a dose of 500 PFU per cell.

#### REFERENCES

1. Wheelock E. P. and I. Tamm, J. exp. Med. v.114, p.33, 1961
2. Levi H. B., Virology v. 22, p 575, 1964
3. Kaverin N. V., Vopr. virusol. no. 1, p.36, 1963
4. Porterfield J. S. and A. C. Allison, Virology v. 10, p. 233, 1960
5. Baltimore D., R. M. Franklin and J. Callender, Biochem. biophys.  
Acta v. 76; p. 3, 1963